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# Decreased Expression of p33ING1 mRNA in Lymphoid Malignancies

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**The ING1 is a newly cloned putative tumor-suppressor gene, which is involved in the p53 signaling pathway. We found decreased expression of ING1 mRNA in 4 of 5 T-cell lines and 5 of 11 B-cell lines including two Burkitt lymphomas and two myelomas. These observations suggest that decreased ING1 expression might play an important role in the development or progression of some lymphoid tumors. Polymerase chain reaction-SSCP and sequencing analyses found neither point mutations nor small deletions in the ING1 gene, suggesting that decreased expression is due to transcriptional or post-transcriptional mechanisms. Am. J. Hematol. 62:118–119, 1999.** © 1999 Wiley-Liss, Inc.

**Key words: p33ING1; p53; 13q34; lymphoma**

## INTRODUCTION

ING1 is a newly cloned candidate tumor-suppressor gene which encodes a family of proteins arising from alternate splicing, including 33-kD nuclear protein (p33ING1). Studies have shown that increased expression of ING1 inhibited cell proliferation, whereas the expression of antisense constructs of ING1 promoted cell transformation. Point mutation of the ING1 gene was found in neuroblastoma cells and reduced expression was observed in breast cancer cell lines [1]. ING1 has been mapped to the long arm of chromosome 13 at q33 to q34 [2], a region in which chromosomal translocations or deletions have been frequently found in lymphoid malignancies. These observations suggest that inactivation of the ING1 gene might be involved in these chromosomal alterations and related to the development and/or disease progression of some lymphoid malignancies. To test this hypothesis, we examined mRNA expression of the ING1 gene in a panel of established cell lines from various lymphoid malignancies.

## MATERIALS AND METHODS

### Cell Lines

CEM, Jurkat, TALL-1, MOLT-4, and MT-2 were T-cell lines and obtained from JCRB (Tokyo, Japan). BALL-1, OZ, KS-1, and BC-1 were non-Burkitt B-cell lines. Daudi, Raji, and KHM10B were derived from Burkitt lymphoma. IM9, RPM18226, U266, and DOBIL-6 were myeloma cell lines. Cells were cultured in RPMI1640 medium with 10% fetal calf serum and performed with passage 3-day intervals.

### Reverse Transcription-Polymerase Chain Reaction Assay of ING1 mRNA Expression

RNA was extracted from each cell line while in the logarithmic growth phase by using the Trizol reagent according to instructions from the manufacturer (Gibco BRL, Tokyo, Japan). cDNA was synthesized from 5 μg of total RNA by using cDNA synthetic kit (Clontech, San Diego, CA). The primer sets for ING1 and GAPDH genes and semiquantitative analysis method for ING1 mRNA expression ("primer-dropping method") were described previously [3].

### PCR-SSCP and cDNA Sequence

PCR reactions were performed by using three pairs of primers as follows: primer-1, 5′-CTGAAGGAGC-

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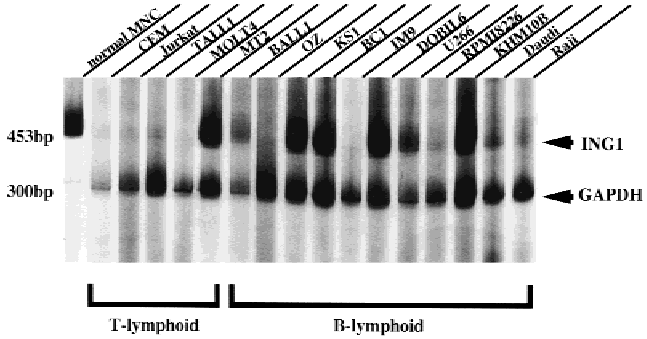


**Fig. 1. Expression of ING1 mRNA in lymphoid malignancies. By using a semiquantitative dropping method, the expression of ING1 and GAPDH mRNA was comparatively examined. In comparison with ING1 mRNA expression of peripheral blood mononuclear cells, a marked decreased in ING1 expression was observed in CEM, Jurkat, TALL-1, MOLT-4, OZ, IM-9, RPMI8226, Daudi, and Raji cell lines. This is a representative figure from three independent experiments.**

TAGACGAGTG-3′ and 5′-GGCTTGTCAGACTGCGCTAC-3′; primer-2, 5′-GTAGCGCAGTCTGACAAGCC-3′ and 5′-ACGCACGAGAAGTGGAACCA-3′; and primer-3, 5′-GACAACGACGAGTGCCCC-3′ and 5′-CTACCTGTTGTAAGCCCTCTCT-3′. PCR products were separated by electrophoresis in 12.5% polyacrylamide gels using GenePhor (Pharmacia Biotech, Tokyo, Japan), and then the bands were purified and sequenced by the direct dideoxy chain termination method using a T7 sequencing kit (Applied Biosystems, Foster, CA).

## RESULTS AND DISCUSSION

The expression of ING1 mRNA was studied in 16 lymphoid cell lines by RT-PCR (Fig. 1). In comparison with that of normal human peripheral blood mononuclear cells, decreased ING1 expression was observed in four (CEM, Jurkat, TALL-1, MOLT-4) of five T-cell lines, all of which were derived from acute lymphocytic leukemia with an immature T-cell phenotype. Decreased expression was also observed in 5 of 11 B-cell lines, including two (Daudi, Raji) of three Burkitt lymphoma and 2 (IM-9, RPMI8226) of 4 myeloma cell lines. Interestingly, about 18% of Burkitt lymphomas have a chromosomal abnormalities at 13q34 [4], where the ING1 gene was mapped. In addition, in the clinical course of multiple myeloma, either partial or complete deletion of chromosome 13 occurs and it is associated with unfavorable prognosis [5]. Although the number of cell lines that we have studied was limited, these observations suggest that ING1 gene might be involved in these diseases.

To elucidate the possible mechanism of reduced ING1 expression, we performed both PCR-SSCP and direct sequencing of the ING1 gene. Neither point mutations nor small deletions were found in the nine cell lines showing decreased ING1 expression (data not shown), suggesting that decreased expression is due to transcriptional or post-transcriptional mechanisms.

Recently, p33ING1 has been reported to physically associate with p53, and to be involved in the p53 signaling pathway. This includes decreasing cell proliferation by modulating p53-dependent transcriptional activation of p21/WAF1 [6,7]. Inhibition of ING1 expression increased the frequency of transformation of NIH3T3 cells as measured by focus formation [1] and prolonged the life span of diploid cultured fibroblasts [3]. Therefore, decreased ING1 expression observed in the present study suggests that inactivation of the ING1 gene plays an important role in the development and/or disease progression of some lymphoid malignancies, especially T-cell leukemias, Burkitt lymphomas, and myelomas. This is the first report examining levels of expression of ING1 mRNA in lymphoid malignancies. Future studies will examine levels of expression of ING1 in lymphoid malignancies harvest from individuals and will determine the mechanism by which these cells depress expression of this putative tumor-suppressor gene product.

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